Remarks

Claims 27-30 are currently pending in this application. Applicants have amended claim 27 herein. Applicants respectfully request reconsideration of the application.

Applicant has amended claim 27 to more distinctly claim and specifically point out the inventive aspect of her invention. Reconsideration of the application is requested on that basis.

35 USC Section 103 Rejection of claims 27-30 over Lizard in view of Bargmann et al.

Claims 27-30 were rejected under 35 USC 103(a) as being obvious over the combination of Lizard et al. in view of Bargmann et al., US Pat. No. 4935341. In particular, the Examiner has stated that Lizard teaches "detecting the chromogenic susbtrate signal using brightfield microscope conditions." Applicants respectfully traverse the rejection, for the following reasons.

Lizard et al., the primary reference, teach methods of detecting HPV DNA *in situ* using biotinylated DNA probes for either chromogenic (EISH) detection using a brightfield microscope, or fluorescence detection using a laser-scanning confocal microscope (LSCM). The focus of Lizard's teaching is on the enhanced detection of fluorescently-detected probes, versus the chromogenically-detected probes, by use of a LSCM in combination with a CCD camera ("The aim of the present study was to detect by FISH low copy numbers of HPV DNA on whole cells," Id, p. 304, col. 1, lines 20-21). However, Lizard and co-workers compared the chromogenic method (EISH) to the LSCM/CCD camera assisted method. They compared the results of both methods on Caski, HeLa and SiHa cell lines, which exhibit differing levels of integrated viral DNA. Caski have 600+ copies of HPV DNA type 16, HeLa 10-50 copies of HPV type 18, and SiHa 1-2 copies of HPV type 16. SiHa is of course the most difficult to detect.

In the EISH method, Lizard et al. could not reliably detect HPV in SiHa cells. "With brightfield microscopy and EISH, hybridization spots were observable in Caski and HeLa cells, but hardly any in SiHa cells." Id, col. 1, lines 13-15. In fact, the last sentence of the Abstract states "Single genes of HPV were visualized most efficiently by association of FISH with LSCM or quantitative microscopy with an intensified CCD camera." *Id.*, p. 303, col. 2, lines 5-8. In the Results section, Lizard et al. go into more detail about their EISH results and state that:

> In SiHa cells, hybridization spots were rare and hardly detectable with the HPV 16 probe (Fig. 1D) . . . In SiHa cells, 1-2 spots were sometimes detectable only in a few cells and their size was in the range of those observed in HeLa cells. *Id*, p. 305, col. 2 lines 4-5; lines 14-16.

Applicant concludes that Lizard et al. is not an enabling reference for showing single-copy detection of HPV DNA using *in situ* hybridization for brightfield detection. It is clear that Lizard et al. may have seen single copies in rare instances, and failed to see single copies in positive (SiHa) cells. Applicant concludes that Lizard may or may not have been detecting HPV chromogenically in SiHa cell lines-it is not proven in light of Lizard et al.'s own equivocal statements.

Applicant does not claim single copy detection of Her-2/neu using LSCM or other fluorescent technique. Applicant's claim has been, and remains even after amendment, directed to "a method of **visually** detecting a **single copy** of the Her-2/neu gene in chromosomal DNA in an intact cell using **brightfield** microscopy." Applicant has added a few words to clarify the detection step of the claim, and now this step reads : "detecting the chromogenic substrate signal visually using conventional brightfield microscope conditions," thus more clearly distinguishing over the teachings of Lizard et al.

Bargmann et al. is cited for teaching a method of detecting the Her-2/neu gene by using a DNA probe to detect point mutations which cause activation of human neu oncogenes. The Examiner combines the method of Lizard et al. and the Her-2/neu probes of Bargman et al. in an attempt to render *prima facie* obvious Applicant's claimed method.

As pointed out above, Lizard et al. do not provide an enabling teaching regarding chromogenic detection of single copies of genes. Instead, Lizard teaches that using LSCM and a CCD camera, one may be able to detect single copies of HPV *in situ* in SiHa cells using fluorescent detection techniques. Lizard admits that they could not reproducibly or reliably detect single copies chromogenically. Therefore, the combination of Lizard et al. and Bargmann et al. fails to make the *prima facie* case alleged by the Examiner.

Applicant respectfully requests reconsideration of the application on the basis of the above arguments and amendments, and believes the application is now in allowable condition.

Respectfully submitted,

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